09/423351

REC'D 17 AUG 1999
WIPO PCT

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
JEC/BP5699806		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB98/01485	22/05/1998	23/05/1997
International Patent Classification (IPC) or national classification and IPC G01N33/68		
Applicant THE INSTITUTE OF CANCER RESEARCH...et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 11 sheets, including this cover sheet.

☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which hav been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 8 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☒ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
- VI ☒ Certain documents cited
- VII ☒ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
08/12/1998	11.08.99
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. (+49-89) 2399-0 Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Authorized officer Knudsen, H Telephone No. (+49-89) 2399

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB98/01485

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-8,10-33,36-66	as originally filed			
9,34,35	as received on	08/12/1998	with letter of	04/12/1998

Claims, No.:

1-42	with telefax of	27/07/1999

Drawings, sheets:

1/19-19/19	as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

see separate sheet

4. Additional observations, if necessary:

see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 39-42.

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 39-42 are so unclear that no meaningful opinion could be formed (*specify*):

see separate sheet

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:

s e s parate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-38
	No:	Claims	
Inventive step (IS)	Yes:	Claims	10,24
	No:	Claims	1-9,11-23,25-38
Industrial applicability (IA)	Yes:	Claims	1-38
	No:	Claims	

2. Citations and explanations

see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

ITEM I:

1.1 The amendments introduced with the new application pages filed with letter of 04.12.1998 does not appear to extend the content of the application beyond what is disclosed in the original application. From original page 5, the reader would know that the CCTδ sequence is disclosed in WO 95/20654 (D1), the application does not state that the sequences in the non-existing Figure 13 should be different from those disclosed in D1.

1.2 However, the use of the term "apical domain" in claim 11 seems to infringe Article 34(2)(b) PCT. The original passages read that CCT complexes may be a CCT microcomplex, a CCT subunit or an active portion thereof. The term "apical domain" appears to be much broader. The original application does not disclose the identification of binding members which bind to a binding site in an apical domain. Claims 11, 13 and 17-19 are therefore examined as if the amendment had not been made.

ITEM III:

3.1 Claim 39 is directed to a domain of a protein and is therefore considered to lack clarity to an extent at which no meaningful opinion on novelty, inventive step and industrial applicability can be given on the subject-matter of claim 39. It is not clear to which extent the term "domain" limits the scope of the claim to a peptide which consists of the amino acid sequence D219 to N394 or whether the claim encompasses all peptides which contain the said sequence. No opinion can be given on claims 40-42 for the same reasons.

ITEM IV:

4. The present set of claims do not appear to be linked by a common inventive concept. Claims 1-19 are directed to methods for identifying a binding member capable of occupying a substrate binding site on the CCT complex. Claims 29-30 concern the use of the binding members in therapy, claims 31- 37 concern a method which involves the use of a binding member and claim 38 is directed to a pharmaceutical composition which contains the binding member .

The identification of the binding members and their use are only considered to be linked by a common concept in case the binding member, per se, is novel and inventive. However, in the present case the binding members, per se, are not considered inventive (cf Item V below). The subject-matter of claims 1-19 is therefore not considered to be linked by a common inventive concept to the subject-matter of claims 29-38.

ITEM V:

NOVELTY:

5.1 The closest prior art is disclosed in the document WO 95/20654 (D1) which discloses 8 different CCT genes and that the corresponding CCT proteins bind to and are capable of folding proteins such as tubulin and actin. Further it is mentioned that antibodies specific for each of the proteins can be made.

5.2 The identification of binding members is not disclosed anywhere in D1 and the subject-matter of claims 1-19 therefore appears to be novel.

5.3 Though a number of proteins which bind to CCT are known, none of the cited prior art documents disclose binding members which occupy a CCT binding site, but are not substrates. Claims 21-28 and 38 therefore appear to be novel. Methods employing the said binding member are considered novel as well and claims 29-37 therefore appear to be novel.

INVENTIVE STEP:

5.4 The skilled person employs as part of his routine work in determining substrate analogues or enzyme inhibitors, the fact that a binding member can be found by measuring inhibition in the binding between a natural substrate and an enzyme in the present of a candidate compound.

5.5 The subject-matter of present claim 1 is distinguished from this common knolwedge in that it is directed to a method for identifying an inhibitor (ie binding member) for CCT binding to its substrate.

The applicant argues that the skilled person would not use the routine technique for identifying binding members for CCT because GroEL, which is the bacterial counterpart of CCT, interacts in an unspecific way with its substrates via hydrophobic regions, and the skilled person in the absence of information as to the toroid structure of CCT would not have started a screening procedure for CCT binding members.

This explanation is, however, contradicted to some extent by the explainations in the introductory part of the present application's description, which states that some proteins are not bound at all by CCT and that CCT may have a specialist actin/tubulin binding role (p.3, l.24-25). Thus, for the skilled person it would be obvious to test whether the binding between CCT and tubulin/actin is inhibited by antibodies binding to certain regions of CCT and by peptide fragments of tubulin/actin. Claims 1-8 therefore do not appear to be inventive.

5.6 Claims 11-15, 17 and 19 do not appear to be inventive for the same reasons as claims 1-8.

5.7 In the search for epitopes, it is a well-known technique in the art to split a peptide into smaller fragments and to test the binding of each of these fragments to the binding partner. The splitting of actin into smaller fragments and testing of the binding of CCT to these fragments (see Figs. 10 & 11) therefore appear to be obvious options for the skilled person.

The applicant argues that this method has been tested in "Biochemistry, vol.38(11), p.3246-3257, (1999)" and leads to the identification of incorrect fragments which are not binding to the CCT binding sites, whereas the structure oriented method, with which the binding member fragments are identified in the present application, gives a correct identification. However, the binding members of claim 20 are defined in general terms and are considered to encompass all actin fragments which show binding to CCT, including the "incorrect" fragments identified in the above scientific article. Claims 20-22 and 25 therefore do not appear to be inventive.

Thus, as regards the fragments mentioned in Figure 10, only those, which show an unexpected strong binding to CCT, are considered inventive. Claims 9, 16 and 23 therefore do not appear to be inventive, whereas claims 10 and 24 appear to be inventive.

5.8 Finally, the modification of a substrate in order to improve its binding affinity is likewise well known in the art. Thus, claim 18 does not appear to be inventive.

5.9 The modifications of the binding member mentioned in claims 26-27 are also well known in the art and therefore does not add anything inventive to claim 20.

5.10 The use of tubulin and actin in medical therapy is widely suggested in the prior art. The use of the active fragments of tubulin and actin would not appear to result in an unexpected effect and claims 28-30 and 38 therefore do not appear to be inventive.

5.11 As mentioned in the top paragraph on p.23 of the present application's description, the design of mimetics is a known approach in the development of pharmaceuticals. Claims 31-37 do not appear to go beyond the design options which the skilled person would use when designing an assay for finding mimetics to the CCT binding substrates.

INDUSTRIAL APPLICABILITY:

5.12 The claims are directed to products and methods, which are carried out in-vitro, and are therefore considered industrially applicable.

P-DOCUMENTS:

5.13 EMBO Journal, vol.16(14), p.4311-4316 published on 16.07.1997

This document is all published after the present application's priority date. It is therefore relevant for only those parts of the present application, if any, which do not have a valid claim to priority. The present written opinion is drafted assuming that the claimed priority is valid.

ITEM VI:

Certain published documents (Rule 70.10)

Application No Patent No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date *(valid claim)* *(day/month/year)*
WO98/13496	02.04.1998	26.09.1997	26.09.1996 & 03.12.1996
WO 98/24909	11.06.1998	03.12.1996	03.12.1996

The above documents are all published after the present application's priority date. They are therefore only relevant for those parts of the present application, if any, which do not have a valid claim to priority. The present written opinion is drafted assuming that the claimed priority is valid.

However, those of the above documents, which enjoy an earlier priority date than the present application, may become relevant prior art in the Regional phase of the present application.

ITEM VII:

It is not possible to incorporate the teaching of a prior art document into the present application's disclosure by the expression "herein incorporated by reference" or equivalents thereof (see p.28 , l.34-35) (cf PCT Guidelines, C-II, 4.17).

ITEM VIII:

8.1 Claims 1 and 11 appear to relate effectively to the same subject-matter and to differ from each other only with regard to the definition of the subject-matter for which protection is sought ..and/or.. in respect of the terminology used for the features of that subject-matter. The aforementioned claims therefore lack conciseness. Moreover, lack of clarity of the claims as a whole arises, since the plurality of independent claims makes it difficult, if not impossible, to determine the matter for which protection is sought, and places an undue burden on others seeking to establish the extent of the protection.

8.2 The same objection applies to claims 5 and 31 which appear to have identical scopes, as well.

8.3 Claim 20 defines the claimed compound by its activity and a size between 5 and 40 amino acids. A functional definition of a compound is allowable only if the definition is unambiguous and it can be tested without undue burden by the skilled person whether or not a given substance falls within the said functional definition. In the present case, the skilled person cannot test whether a given substance occupies a CCT substrate binding site. Moreover, the document "Biochemistry, vol.38(11), p.3246-3257, (1999)" shows that some uncertainty exists in the field. The binding affinity of compounds often differ by degree. In the present application, it is not clear to what degree the test substance must occupy the binding site.

Article 34

subtrates and then release them again. It may be preferable to obtain a binding member that binds tightly to the CCT complex or part thereof so that it is not released in the normal way. Again modification of the peptides or peptide fragments may be carried out in order to achieve optimum binding characteristics.

Further, such peptides may be coupled with a coupling partner, preferable a second peptide derived from other than a substrate of CCT, to form of a fusion protein. Such second peptide may provide other characteristics such as the ability to cross a cell membrane so as to deliver the binding members into the cytoplasm.

~~The present invention further provides polypeptides~~ comprising a CCT substrate binding site or active portion thereof. Preferably, said polypeptide will comprise an amino acid sequence having at least 80% homology with any one of the sequences for CCT apical domain residues even more preferably an amino acid sequence having at least 90% or 95% homology therewith.

Such CCT substrate binding sites or their active portions may be used in assays for screening for further binding ~~members capable of modulating the interaction of a protein~~ to be folded and the CCT complex. These binding members, as mentioned above, are preferably peptides and may be useful as peptide mimetics to inhibit the interaction of the CCT complex and the protein to be folded. Examples of such binding members include antibodies which may be raised against specific CCT substrate binding sites according to well known techniques. Such antibodies form a further aspect of the present invention and are described in more detail below.

Figure 13 shows in graphical form the absorbance at 410nm for the peptides as illustrated in Table 2 in order to show that actin makes contacts with isolated CCTδ subunit apical domain.

Figure 14 shows in graphical form the absorbance at 410nm for the peptides as illustrated in Table 2 to show that GroEl recognises the same actin peptide sequences as CCT but also recognises several others in addition.

Detailed description

Definitions

"CCT" shall mean the complex comprising CCT subunits α, β, γ, δ, ϵ, ζ, η and θ in the form of a single or double toroid structure described in Kubota et al, Eur J. Biochem (1995) 230 , p3-16.

Parts of the CCT complex are described below as a CCT micro-complex, a CCT subunit, or an active portion of a CCT subunit.

"CCT micro-complex" shall mean any combination of two or more CCT subunits.

"CCT subunit" shall mean any individual protein encoded by one of the CCT genes Ccta (CCT1), Cctb (CCT2), Cctc (CCT3), Cctd (CCT4), Ccte (CCT5), CctZ1, CctZ2 (CCT6), Ccth (CCT7) or Cctq (CCT8) described in Kubota et al , Eur. J. Biochem. (1995) 230 , p3-16; Kubota et al, Gene (1995) 154 231-236; Kubota et al FEBS LETTERS (1997) 402 53-56.

Claims

1) Use of a CCT complex or part thereof for identifying a binding member capable of occupying a substrate binding site on the CCT complex or part thereof wherein the binding member inhibits the binding of the CCT substrate and the CCT complex.

2) Use according to claim 1 wherein the binding member is an antibody.

3) Use according to claim 1 wherein the binding member is a peptide or a peptide fragment.

4) Use according to claim 3 wherein the peptide or peptide fragment is greater than 5 amino acids in length.

5) Use according to claim 4 wherein the peptide or peptide fragment is from 5 to 40 amino acids in length.

6) Use according to any one of claims 3 to 5 wherein the peptide or peptide fragment is derived from a CCT substrate.

7) Use according to claim 6 wherein the substrate is selected from the group consisting of actin, tubulin or cyclin.

8) Use according to claim 7 wherein the substrate is actin.

9) Use according to any one of claims 3 to 8 wherein the peptide or peptide fragments comprises any one of the sequences shown in Figure 10.

10) Use according to any one of claims 3 to 9 wherein the peptide or peptide fragment comprises the amino acid sequence GRPRH.

11) A method of identifying a binding member capable of occupying a substrate binding site on a CCT apical domain; comprising the steps of

contacting a candidate binding member with said CCT apical domain; and

determining binding between said candidate binding member and the CCT apical domain.

12) A method according to claim 11 wherein the binding member is a peptide or peptide fragment.

13) A method according to claim 11 or claim 12 wherein the candidate binding member is a peptide or peptide fragment having an amino acid sequence corresponding to the amino acid sequence of a CCT apical domain.

14) A method according to claim 13 wherein the CCT substrate is actin.

15) A method according to claim 14 wherein the CCT substrate is tubulin.

16) A method according to any one of claims 12 to 14 wherein the peptide or peptide fragment comprises any one of the sequences as shown in Fig. 10.

17) A method according to any one of claim 11 to 16 further comprising the step of immobilising the candidate binding member on a solid phase prior to contacting with the CTT apical domain.

18) A method according to any one of claims 11 to 17 further comprising the step of modifying the candidate binding member to improve its binding with the CCT apical domain.

19) A method according to any one of claim 11 to 18 wherein binding between the candidate binding member and the CCT apical domain is determined by a competitive assay.

20) A binding member capable of occupying a CCT substrate binding site, comprising of an amino acid sequence of 5 to 40 amino acids derived from a CCT substrate.

~~21) A binding member according to claim 20 wherein the~~ CCT substrate is selected from the group consisting of actin, tubulin or cyclin.

22) A binding member according to claim 21 wherein the CCT substrate is actin.

23) A binding member according to claim 22 comprising any one of the amino acid sequences as shown in Fig. 10.

~~24) A binding member according to claim 23 comprising the~~ amino acid sequence GRPRH

25) A binding member according to any one of claims 20 to 24 for use in binding to a CCT complex such that it blocks a substrate binding site on said CCT complex thereby effecting the biological activity of the CCT complex.

26) A binding member according to any one of claims 20 to 25 linked to a coupling partner.

27) A binding member according to claim 26 wherein the coupling partner is a second peptide and the binding member and the second peptide form a fusion protein.

28) A binding member according to any one of claims 20 to claim 27 for use in medical treatment.

29) Use of a binding member according to any one of claims 20 to 27 in the preparation of a medicament for the treatment of cancer cells wherein the medicament is administered to said cells to effect the biological activity of a CCT complex within the cell.

30) Use according to claim 39 wherein the medicament further comprises a cancer drug.

31) A method for screening for mimetics of binding members according to any one of claims 20 to 27 comprising exposing said binding members and a candidate mimetic to a CCT substrate binding site or active portion thereof, so that the candidate mimetic and the binding member compete to bind to the CCT substrate binding site; and detecting the extent of binding of the candidate mimetic or the binding member to the CCT substrate binding site.

32) A method according to claim 31 further comprising screening the candidate mimetics for biological activity.

33) A method according to claim 32 wherein the biological activity is the inhibition of cytoskeletal assembly.

34) A method according to claim 32 wherein the biological activity is CCT complex dis-assembly.

35) A method according to any one of claims 27 to 34 wherein the binding member or the candidiate mimetic is immobilised on a solid support.

36) A method according to any one of claims 31 to 35 wherein the extent of binding of the candidate mimetic is detected by labelling the CCT substrate binding site complex or active portion thereof or by using a labelled antibody capable of binding to the CCT substrate binding domain.

37) A method according to any one of claims 31 to 36 wherein the CCT substrate binding site comprises the sequence corresponding to residues D219 to N394 of CCTδ.

38) A pharmaceutical composition comprising a binding member according to any one of claim 20 to 28 in combination with a pharmaceutically acceptable carrier.

39) A CCT apical domain having at least 80% homology with the amino acid sequence of D219 to N394 of CCTδ.

40) A nucleic acid molecule encoding the polypeptide according to claim 39.

41) A vector comprising the nucleic acid according to claim 40.

42) A host cell comprising the vector according to claim 41 or the nucleic acid according to claim 40.

TENT COOPERATION TREATY

PCT

09/423351

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
JEC/BP5699806		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 98/ 01485	22/05/1998	23/05/1997
Applicant		
THE INSTITUTE OF CANCER RESEARCH...et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [X] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title,**
   - [X] the text is approved as submitted by the applicant
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   - [X] the text is approved as submitted by the applicant
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

   Figure No. _____
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [X] None of the figures.

ernational application No.

INTERNATIONAL SEARCH REPORT

PCT/GB 98/01485

Box I Obs rvations where certain claims were f und unsearchable (Continuati n f it m 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 38, 40-44
because they relate to subject matter not required to be searched by this Authority, namely:
A search was made for the general concept of polypeptides arising from the CCT substrate binding site but due to the lack of specific nucleic acid sequence data arising from the absence of figure 13, the precise scope of the claim could not be searched.

2. [X] Claims Nos.: 38, 40-44
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
The claims rely upon sequence data found in figure 13, Figure 13 does not appear to have been filed with te documents. The relevant sequences could not be indentified through the filed sequence listings either.

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 98/01485

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/68 C07K14/47 C07K16/18 C07H21/00

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N C07K C12P C07H

Documentation searched other than minimumdocumentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 98 24909 A (MEDICAL RES COUNCIL ;FERSHT ALAN ROY (GB); ZAHN RALPH (GB); ALTAMI) 11 June 1998 see the whole document ---	1-47
P,X	WO 98 13496 A (MEDICAL RES COUNCIL ;FERSHT ALAN ROY (GB); ZAHN RALPH (GB); ALTAMI) 2 April 1998 see the whole document ---	1-47
X	WO 95 20654 A (CANCER RES INST ROYAL ;WILLISON KEITH ROBERT (GB); KUBOTA HIROSHI) 3 August 1995 cited in the application	40-44
A	see claims --- -/--	1-44

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of theinternational search	Date of mailing of the international search report
9 October 1998	22/10/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Routledge, B

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 25681 A (UNIV NEW YORK) 23 December 1993	40-44
A	see the whole document	1-39, 45-47

X	GB 2 270 076 A (UNIV MANCHESTER) 2 March 1994	40-44
A	see the whole document	1-39, 45-47

P,X	LIOU A K ET AL: "Elucidation of the subunit orientation in CCT (chaperonin containing TCP1) from the subunit composition of CCT micro- complexes." EMBO JOURNAL, (1997 JUL 16) 16 (14) 4311-6. JOURNAL CODE: EMB. ISSN: 0261-4189., XP002079764 ENGLAND: United Kingdom see the whole document	1-47

A	FRYDMAN J ET AL: "Chaperones get in touch: the Hip-Hop connection" TIBS TRENDS IN BIOCHEMICAL SCIENCES, vol. 22, no. 3, March 1997, page 87-92 XP004055066 see the whole document	1-47

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 98/01485

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9824909 A	11-06-1998	AU 1036397 A	29-06-1998
		AU 4467497 A	17-04-1998
		WO 9813496 A	02-04-1998
WO 9813496 A	02-04-1998	WO 9824909 A	11-06-1998
		AU 4467497 A	17-04-1998
		AU 1036397 A	29-06-1998
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		CA 2182499 A	03-08-1995
		EP 0742822 A	20-11-1996
		JP 10500001 T	06-01-1998
WO 9325681 A	23-12-1993	NONE	
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		EP 0656945 A	14-06-1995
		EP 0861892 A	02-09-1998
		WO 9404676 A	03-03-1994
		JP 8500016 T	09-01-1996
		US 5777083 A	07-07-1998

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 98/01485

Box I Observations where certain claims were found unsearchable (Continuation of item 1 f first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 38, 40-44
because they relate to subject matter not required to be searched by this Authority, namely:
A search was made for the general concept of polypeptides arising from the CCT substrate binding site but due to the lack of specific nucleic acid sequence data arising from the absence of figure 13, the precise scope of the claim could not be searched.

2. [X] Claims Nos.: 38, 40-44
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
The claims rely upon sequence data found in figure 13, Figure 13 does not appear to have been filed with te documents. The relevant sequences could not be indentified through the filed sequence listings either.

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule6.4(a).

B x II Observations where unity of invention is lacking(Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invitepayment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
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Crystal Plaza 2
Washington, DC 20231
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in its capacity as elected Office

Date of mailing (day/month/year) 20 April 1999 (20.04.99)	
International application No. PCT/GB98/01485	**Applicant's or agent's file reference** JEC/BP5699806
International filing date (day/month/year) 22 May 1998 (22.05.98)	**Priority date** (day/month/year) 23 May 1997 (23.05.97)
Applicant WILLISON, Keith et al	

1. The designated Office is hereby notified of its election made:

   [X] in the demand filed with the International Preliminary Examining Authority on:

   08 December 1998 (08.12.98)

   [ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

   [ ] was not

   made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

S. Mafla

Telephone No.: (41-22) 338.83.38

WO 98/53322
PCT/GB98/01485

F ENT COOPERATION TREA

09/423351

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:
KIDDLE, Simon, J.
Mewburn Ellis
York House
23 Kingsway
London WC2B 6HP
ROYAUME-UNI

Date of mailing (day/month/year) 26 November 1998 (26.11.98)		
Applicant's or agent's file reference JEC/BP5699806		**IMPORTANT NOTICE**
International application No. PCT/GB98/01485	**International filing date** (day/month/year) 22 May 1998 (22.05.98)	**Priority date** (day/month/year) 23 May 1997 (23.05.97)
Applicant THE INSTITUTE OF CANCER RESEARCH: ROYAL CANCER HOSPITAL et al		

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   AU,CA,EP,JP,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   None

   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   26 November 1998 (26.11.98) under No. WO 98/53322

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO
34, chemin des Colombettes
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Authorized officer
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Continuation of Form PCT/IB/308

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

Date of mailing (day/month/year)	IMPORTANT NOTICE
26 November 1998 (26.11.98)	
Applicant's or agent's file reference	**International application No.**
JEC/BP5699806	PCT/GB98/01485

The applicant is hereby notified that, at the time of establishment of this Notice, the time limit under Rule 46.1 for making amendments under Article 19 has not yet expired and the International Bureau had received neither such amendments nor a declaration that the applicant does not wish to make amendments.

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subtrates and then release them again. It may be preferable to obtain a binding member that binds tightly to the CCT complex or part thereof so that it is not released in the normal way. Again modification of the peptides or peptide fragments may be carried out in order to achieve optimum binding characteristics.

Further, such peptides may be coupled with a coupling partner, preferable a second peptide derived from other than a substrate of CCT, to form of a fusion protein. Such second peptide may provide other characteristics such as the ability to cross a cell membrane so as to deliver the binding members into the cytoplasm.

The present invention further provides polypeptides comprising a CCT substrate binding site or active portion thereof. Preferably, said polypeptide will comprise an amino acid sequence having at least 80% homology with any one of the sequences for CCT apical domain residues as shown in Figure 13, even more preferably an amino acid sequence having at least 90% or 95% homology with any one of the sequences shown in Figure 13.

Such CCT substrate binding sites or their active portions may be used in assays for screening for further binding members capable of modulating the interaction of a protein to be folded and the CCT complex. These binding members, as mentioned above, are preferably peptides and may be useful as peptide mimetics to inhibit the interaction of the CCT complex and the protein to be folded. Examples of such binding members include antibodies which may be raised against specific CCT substrate binding sites according to well known techniques. Such antibodies form a further aspect of the present invention and are described in more detail below.

all lanes, CCT was incubated with peptide on ice for one hour. Samples were electrophoresed on 6% native gels, transferred to nitrocellulose membrane and incubated with Neutravidin-HRP (Pierce) at 2µg per ml to reveal the distribution of biotinylated peptides. The arrowed region (Figure 11B) shows CCT complexes bound by peptides. Figure 11C shows the results quantitated.

Figure 12 shows the interaction of cyclin D1 and cyclin E with CCT. p Bluescript plasmids containing full length mouse-cyclin D1 cDNA or human cyclin E cDNA were used to programme rabbit reticulocyte lysate transcription translation systems (Liou & Willison, EMBO J. 16, 4311-4316, 1997). Time courses of interactions of cyclins with CCT were analysed on 6% native polyacrylamide gels (Liou & Willison,EMBO J. 16, 4311-4316, 1997). At the indicated times, 5µl aliquots of the lysate reactions were added to 7 µl of f10ml EDTA (ph 8.0) and 4 µl of 4x gel loading buffer and placed on ice. The lanes 1 - 6 show CCT α at t = 0, 5, 10, 20, 30, 60 minutes. Lanes 7 - 12 show pBSK CY1 1 (mouse D1) at t = 0, 5,10, 20, 30, 60 minutes. The right hand panel (lanes 13 - 18) shows a time course expression of cyclin E at t = 0, 5, 10, 20, 30, 60 minutes. In the right hand panel, the lane marked M shows the migration of molecular weight markers of 886kDa and 43 kDa. This kinetic analysis shows that cyclins do not appear to be interacting with CCT in a manner resembling bone fide substrates, such as actins and tubulins, but seem to have similar kinetics as the cycling of CCT subunits into rabbit CCT in the lysate. This suggests some regulatory role for the interactions of cyclins with CCT.

Figure 13 shows mouse and Saccharomyces cerevisiae CCT apical domain squences CCT1 - CCT8. The amino acid and nucleotide sequence of CCTδ residues D219 -N394 is marked.

Figure 14 shows in graphical form the absorbance at 410nm for the peptides as illustrated in Table 2 in order to show that actin makes contacts with isolated CCTδ subunit apical domain.

Figure 15 shows in graphical form the absorbance at 410nm for the peptides as illustrated in Table 2 to show that Groel recognises the same actin peptide sequences as CCT but also recognises several others in addition.

Detailed description

Definitions

"CCT" shall mean the complex comprising CCT subunits α, β, γ, δ, ε, ζ, η and θ in the form of a single or double toroid structure described in Kubota et al, Eur J. Biochem (1995) 230 , p3-16.

Parts of the CCT complex are described below as a CCT micro-complex, a CCT subunit, or an active portion of a CCT subunit.

"CCT micro-complex" shall mean any combination of two or more CCT subunits.

"CCT subunit" shall mean any individual protein encoded by one of the CCT genes Ccta (CCT1), Cctb (CCT2), Cctc (CCT3), Cctd (CCT4), Ccte (CCT5), CctZ1, CctZ2 (CCT6), Ccth (CCT7) or Cctq (CCT8) described in Kubota et al , Eur. J. Biochem. (1995) 230 , p3-16; Kubota et al, Gene (1995) 154 231-236; Kubota et al FEBS LETTERS (1997) 402 53-56.

Claims

1) Use of a CCT complex or part thereof for identifying a binding member capable of occupying a substrate binding site on the CCT complex or part thereof.

2) Use according to claim 1 wherein the binding member inhibits the binding of the CCT substrate and the CCT complex.

3) Use according to claim 1 or claim 2 wherein the binding member is a peptide or a peptide fragment.

4) Use according to claim 3 wherein the peptide or peptide fragment is greater than 5 amino acids in length.

5) Use according to claim 4 wherein the peptide or peptide fragment is from 5 to 40 amino acids in length.

6) Use according to any one of claims 3 to 5 wherein the peptide or peptide fragment is derived from a CCT substrate.

7) Use according to claim 6 wherein the substrate is selected from the group consisting of actin, tubulin or cyclin.

8) Use according to claim 7 wherein the substrate is actin.

9) Use according to any one of claims 3 to 8 wherein the peptide or peptide fragments comprises any one of the sequences shown in Figure 10.

10) Use according to any one of claims 3 to 9 wherein the

peptide or peptide fragment comprises the amino acid sequence GRPRH.

11) A method of identifying a binding member capable of occupying a substrate binding site on a CCT complex or part thereof; comprising the steps of

contacting a candidate binding member with said CCT complex or part thereof; and

determining binding between said candidate binding member and the CCT complex or part thereof.

12) A method according to claim 11 wherein the binding member is a peptide or peptide fragment.

13) A method according to claim 11 or claim 12 wherein the candidate binding member is a peptide or peptide fragment having an amino acid sequence corresponding to the amino acid sequence of a CCT substrate.

14) A method according to claim 13 wherein the CCT substrate is actin.

15) A method according to claim 14 wherein the CCT substrate is tubulin.

16) A method according to any one of claims 12 to 14 wherein the peptide or peptide fragment comprises any one of the sequences as shown in Fig. 10.

17) A method according to any one of claim 11 to 16 further comprising the step of immobilising the candidate binding member on a solid phase prior to contacting with the CTT complex or part thereof.

18) A method according to any one of claims 11 to 17

wherein the CCT complex or part thereof is a CCT micro-complex, a CCT subunit or active portion thereof.

19) A method according to any one of claims 11 to 18 further comprising the step of modifying the candidate binding member to improve its binding with the CCT complex or part thereof.

20) A method according to any one of claim 11 to 19 wherein binding between the candidate binding member and the CCT complex is determined by a competitive assay.

21) A binding member capable of occupying a CCT substrate binding site, comprising of an amino acid sequence of 5 to 40 amino acids derived from a CCT substrate.

22) A binding member according to claim 21 wherein the CCT substrate is selected from the group consisting of actin, tubulin or cyclin.

23) A binding member according to claim 22 wherein the CCT substrate is actin.

24) A binding member according to claim 23 comprising any one of the amino acid sequences as shown in Fig. 10.

25) A binding member according to claim 24 comprising the amino acid sequence GRPRH

26) A binding member according to any one of claims 21 to 25 for use in binding to a CCT complex such that it blocks a substrate binding site on said CCT complex thereby effecting the biological activity of the CCT complex.

27) A binding member according to any one of claims 21 to 26 linked to a coupling partner.

28) A binding member according to claim 27 wherein the coupling partner is a second peptide and the binding member and the second peptide form a fusion protein.

29) A binding member according to any one of claims 21 to claim 28 for use in medical treatment.

30) Use of a binding member according to any one of claims 21 to 28 in the preparation of a medicament for the treatment of cancer cells wherein the medicament is administered to said cells to effect the biological activity of a CCT complex within the cell.

31) Use according to claim 30 wherein the medicament further comprises a cancer drug.

32) A method for screening for mimetics of binding members according to any one of claims 21 to 28 comprising exposing a binding member according to any one of claims 21 to 28 and a candidate mimetic to a CCT substrate binding site or active portion thereof, so that the candidate mimetic and the binding member compete to bind to the CCT substrate binding site, and detecting the extent of binding of the candidate mimetic or the binding member to the CCT substrate binding site.

33) A method according to claim 32 further comprising screening the candidate mimetics for biological activity.

34) A method according to claim 33 wherein the biological activity is the inhibition of cytoskeletal assembly.

35) A method according to claim 33 wherein the biological activity is CCT complex dis-assembly.

36) A method according to any one of claims 28 to 35 wherein the binding member or the candidiate mimetic is immobilised on a solid support.

37) A method according to any one of claims 32 to 36 wherein the extent of binding of the candidate mimetic is detected by labelling the CCT substrate binding site complex or active portion thereof or by using a labelled antibody capable of binding to the CCT substrate binding domain.

38) A method according to any one of claims 32 to 37 wherein the CCT substrate binding site comprises the sequence corresponding to residues D219 to N395 of CCTδ as shown in Figure 13.

39) A pharmaceutical composition comprising a binding member according to any one of claim 21 to 29 in combination with a pharmaceutically acceptable carrier.

40) A polypeptide comprising a specific CCT substrate binding site comprising an amino acid sequence having at least 80% homology with any one of the CCT apical domains as shown in Figure 13.

41) A polypeptide comprising a specific CCT substrate binding site according to claim 40 having at least 80% homology with the the amino acid sequence of D219 to N394 as shown in Figure 13.

42) A nucleic acid molecule encoding the polypeptide according to claim 40 or claim 41.

43) A vector comprising the nucleic acid according to claim 42.

44) A host cell comprising the vector according to claim 43 or the nucleic acid according to claim 42.

45) An antibody specific for a binding member according to any one of claims 21 to 29.

46) A method of identifying binding members capable of occupying a CCT substrate binding site, said method comprising the step of screening a peptide library with an antibody according claim 45 and detecting binding between said antibody and a peptide.

47) A method according to claim 46 further comprising the step of isolating said peptide and determining its ability to bind to CCT complex or part thereof.